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Forty years of genetic recombination in bacteria

Between April and June 1946, Joshua Lederberg and Edward L. Tatum carried out a series of experiments that proved that bacteria can exchange their genes by sexual crossings. The experiments were reported in Nature *just 40 years ago*[1]*. In the following pair of articles, Joshua Lederberg first provides a personal reminiscence of the circumstances of the discovery and then, together with Harriet Zuckerman, considers it as a possible case of 'postmature' scientific discovery.*

A fortieth anniversary reminiscence

Joshua Lederberg

In September 1941, when I started as an undergraduate at Columbia University, the genetics of bacteria was still a no-man's-land between the disciplines of genetics and (medical) bacteriology. The question whether "bacteria have genes, like all other organisms" was still unanswered, indeed rarely asked. My own thoughts at that moment lay elsewhere. I looked forward to a career in medical research applying chemical analysis to problems like cancer and the malfunctions of the brain. Cytotoxicology then appeared to be the most promising approach to cell biochemistry. It was Francis J. Ryan (d. 1963) who turned my attention to the sharper tools of genetics.

Ryan had spent 1941–42 as a postdoctoral fellow at Stanford University, where he had met G. W. Beadle and E. L. Tatum (d. 1975), and had become fascinated with their recent invention of nutritional mutations in *Neurospora* as a tool for biochemical genetic analysis[2]. Although working on a fungus like *Neurospora* did not go down smoothly in a Department of Zoology as at Columbia, where Ryan had accepted an instructorship, he established a laboratory to continue these studies. In January 1943 I was fortunate to get a job in his laboratory assisting in the preparation of media and handling of *Neurospora* cultures. Ryan's personal qualities as a teacher and the setting of serious research, discussion with him, other faculty members and graduate students in the department nourished my education as a scientist. On 1 July 1943, I was called to active duty in the United States Naval Reserve, and my further months at Columbia College alternated with spells of duty at the United States Naval Hospital, St Albans, Long Island. There, in the clinical parasitology laboratory, I had abundant opportunity to observe the life cycle of *Plasmodium vivax*. This experience dramatized the sexual stages of the malaria parasite, which undoubtedly sensitized me to the possibility of cryptic sexual stages in other microbes (perhaps even bacteria). In October 1944,

Lederberg in 1945

I was reassigned to begin my studies at Columbia Medical School; but I continued working with Ryan at the Morningside Heights campus.

Discovery

The important biological discovery of that year, by Avery, MacLeod and McCarty, was the identification of DNA as the substance responsible for the *Pneumococcus* transformation[3]. This phenomenon could be viewed as the transmission of a gene from one bacterial cell to another; but such an interpretation was inevitably clouded by the obscure understanding of bacterial genetics at the time. Avery's work, at the Rockefeller Institute in New York, was promptly communicated to Columbia biologists by Theodosius Dobzhansky (who visited Rockefeller) and by Alfred Mirsky (of the Rockefeller faculty) who was a close collaborator of Arthur Pollister in the Zoology Department. The work was the focus of widespread and critical discussion among the faculty and students. Mirsky was a vocal critic of the purported chemical identification of the transforming agent, while applauding the central importance of the work. For my own part, the transcendent leap was simply the feasibility of knowing the chemistry of the gene. Whether this was DNA or protein would certainly be clarified quickly, provided the *Pneumococcus* transformation could be securely retained within the conceptual domain of gene transmission. I read the Avery, MacLeod and McCarty paper on 20 January 1945, prompted by Harriett Taylor (later Ephrussi-Taylor) a graduate student in Zoology who planned to pursue her postdoctoral studies with Avery. My excited response is recorded as ... "unlimited in its implications... Direct demonstration of the multiplication of transforming factor... Viruses are gene-type compounds."

At once, I thought of attempting similar transformations by DNA *in Neurospora*. This organism had a well understood life-cycle and genetic structure. The biochemical mutants opened up by Beadle and Tatum also allowed the efficient detection of nutritionally self-sufficient (prototrophic) forms, even if these were vanishingly rare. This would facilitate the assay of transformational events.

Between January and May, 1945, I shared this idea with Francis Ryan; in June, he invited me to work on the subject with him. To our dismay, we soon discovered that the leucine-minus *Neurospora* mutant would spontaneously revert to prototrophy[4], leaving us with no reliable assay for the effect of DNA in mediating genetic change in *Neurospora*. Questions about the biology of transformation would remain inaccessible to conventional genetic analysis if bacteria lacked a sexual stage. But was it true that bacteria were asexual? Rene Dubos' monograph, *The Bacterial Cell*[5], footnoted how inconclusive the claims were for or against any morphological exhibition of sexual union between bacterial cells.

My notes dated 8 July 1945 detail hypothetical experiments both to search for mating among *Monilia* (medically important yeast-like fungi) and to seek genetic recombination in bacteria (by the protocol that later proved to be successful). These notes coincide with the beginning of my course in medical bacteriology. They were provoked by the contrast of the tra-

ditional teaching that bacteria were *Schizomycetes*, asexual primitive plants, with an appreciation of sexuality in yeast[6], which was represented at Columbia by the graduate research work of Sol Spiegelman and Harriett Taylor.

Dubos[5] cited many unclear, and two clear-cut negative results[7,8] for sexuality in bacteria using genetic exchange methodology. But these two studies had no selective method for the detection of recombination and so would have overlooked the process had it occurred less often than perhaps once per thousand cells. With the use of a pair of nutritional mutants, say A^+B^- and A^-B^+, one could plate out innumerable cells in a selective medium and find a single A^+B^+ recombinant. In early July, I began experiments along these lines. In the first instance I used a set of biochemical mutants in *Escherichia coli*, which I began to accumulate in Ryan's laboratory. To avoid the difficulty that had arisen in our *Neurospora* experiments, a spontaneous reversion from A^-B^+ to A^+B^+, the strategy would be to use a pair of double mutants: $A^-B^-C^+D^+$ and $A^+B^+C^-D^-$. Sexual crossing should still generate $A^+B^+C^+D^+$ prototroph recombinants. These would be unlikely to arise by spontaneous reversions which, in theory, requires the coincidence of two rare events; $A^- \rightarrow A^+$ and $B^- \rightarrow B^+$. Much effort was devoted to control experiments to show that double reversions would follow this model, and so occur at a negligible frequency in the cultures handled separately. Thus the occurrence of prototrophs in the mixed cultures would be presumptive evidence of genetic recombination.

Long shot

Meanwhile at Stanford, Ed Tatum, whose doctoral training at Wisconsin had been in the biochemistry of bacteria, was returning to bacteria as experimental subjects, having published two papers on the production of biochemical mutants in *E. coli*[9], including double mutants like those described here. During the summer of 1945 Francis Ryan learned that Tatum was leaving Stanford to set up a new programme in microbiology at Yale. He suggested that, rather than merely ask Tatum to share these new strains, I apply to work with him and get the further benefit of his detailed experience and general wisdom. Tatum agreed and suggested that I arrive in New Haven in late March, to give him time to set up his laboratory. He hinted that he had some similar ideas of his own, but never elaborated them. The arrangement suited him by leaving him free to complete his work on the biochemistry of *Neurospora*, perform the heavy administrative duties of his new programme, and still participate in the long-shot gamble of looking for bacterial sex.

Experimental luck

1. We have learned[12] that *E. coli* strain K–12 itself was a remarkably lucky choice of experimental material: only about one in twenty randomly chosen strains of *E. coli* would have given positive results in experiments designed according to our protocols. In particular, strain B, which has become the standard material for work on bacteriophage, would have been stubbornly unfruitful. Tatum had acquired K–12 from the routine stock culture collection in Stanford's microbiology department when he sought an *E. coli* strain to use as a source of tryptophanase in work on tryptophan synthesis in *Neurospora*[13]. The same strain was then in hand when he set out to make single, and then double mutants in *E. coli*[9]. In 1946, I was very much aware of strain specificities and was speculating about mating types (as in *Neurospora*). I have no way to say how many other strains would have been tried, or in how many combinations, had the June 1946 experiments not been successful.

2. An equally important piece of luck was that, the selected markers Thr (threonine) and Leu (leucine) are found almost at the origin of the *E. coli* chromosome map[14]. The cognoscenti will recognize that in a cross $B^-M^-T^+L^+F^+ \times B^+M^+T^-L^-F^-$, the configuration used in June 1946, these chromosome localizations offer almost a maximum yield of selectible recombinants. We were therefore led stepwise into the complexities of mapping.

It took about six weeks, from the first serious efforts at crossing in mid-April 1946, to establish well-controlled, positive results. These experiments could be done overnight, so the month of June allowed over a dozen repetitions, and the recruitment of almost a dozen genetic markers in different crosses. Besides the appearance of $A^+B^+C^+D^+$ prototrophs, it was important to show that additional unselected markers in the parent stocks would segregate and recombine freely in the prototrophic progeny. This result left little doubt as to the interpretation of the experiments.

An immediate opportunity for public announcement presented itself at the international Cold Spring Harbor Symposium in July. This was dedicated to the genetics of microorganisms, signalling the postwar resumption of major research in a field that had been invigorated by the new discoveries with *Neurospora*, phage, and the role of DNA in the *Pneumococcus* transformation. Tatum was already scheduled to talk about his work on *Neurospora*. We were granted a last-minute improvisation in the schedule to permit a brief discussion of our new results.

The discussion was lively. The most principled criticism came from Andre Lwoff who worried about cross-feeding of nutrients between the two strains without their having in fact exchanged genetic information. Having taken great pains to control this possibility, I felt that the indirect genetic evidence was quite conclusive. Fortunately, Max Zelle mediated the debate, and generously offered to advise and assist me in the direct isolation of single cells under the microscope. These subsequent observations did quiet remaining concerns of the group that Lwoff had assembled at the Pasteur Institute, including Jacques Monod, Francois Jacob and Elie Wollman, who were to make the most extraordinary contributions to the further development of the field. The single cell methods were also useful in later investigations in several fields. A direct result of the Cold Spring Harbor meeting was the prompt ventilation of all the controversial issues. With a few understandable, but minor points of resistance, genetic recombination in bacteria was soon incorporated into the mainstream of the burgeoning research in molecular biology, and after another decade or so into the standard texts of bacteriology. It still took some years to work out the intimate details of crossing in *E. coli*; some, including the crucial question of the physical mechanism of DNA transfer between mating cells, are still obscure.

The public image of the scientific fraternity today has seldom been so problematic and the system cannot avoid putting a high premium on competition and self-assertion. We can recall with gratification how the personalities of Ryan[10] and Tatum[11] exemplified norms of nurture, dignity, respect for others, and above all a regard for the advance of knowledge.

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1. Lederberg, J. & Tatum, E.L. *Nature* **158**, 558 (1946).
2. Beadle, G.W. & Tatum, E.L. *Proc. natn. Acad. Sci. U.S.A.* **27**, 499 – 506 (1941).
3. Avery, O.T., MacLeod, C.M. & McCarty, M. *J. exp. Med.* **79**, 137 – 158 (1944).
4. Ryan, F.J. & Lederberg, J. *Proc. natn. Acad. Sci. U.S.A.* **32**, 163–173 (1946).
5. Dubos, R. *The Bacterial Cell* (Harvard, Cambridge, 1945).
6. Winge & Lausten, O. *C.R. Lab. Carlsberg, Ser. physiol.*, **22**, 99-119, (1937).
7. Sherman, J.M. & Wing, H.U. *J. Bact.* **33**, 315 – 321 (1937).
8. Gowen, J.W. & Lincoln, R.E. *J. Bact.* **44**, 551 – 554 (1942).
9. Gray C.H. & Tatum, E.L. *Proc. natn. Acad. Sci. U.S.A.* **30**, 404–410 (1944).
10. Lederberg, J. *in University on the Heights*, (ed. First, W.) 105 – 109. (Doubleday, Garden City, New York, 1969).
11. Lederberg, J. *A. Rev. Genet.* **13**, 1–5 (1979).
12. Lederberg J. *Science* **114**, 68 – 69 (1951).
13. Tatum, E.L. & Bonner, D.M. *Proc. natn. Acad. Sci. U.S.A.* **30**, 30 – 37 (1944).
14. Bachmann, B.J. *Microb. Revs.* **47**, 180 – 230 (1983).

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